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Prey availability and abiotic requirements of immature stages of the aphid predator *Sphaerophoria rueppellii*

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14 Abstract: Aphidophagous hoverflies (Diptera: Syrphidae) are natural enemies with a high 15 potential as biological control agents. Nevertheless, there is only one commercially available 16 species, *Episyrphus balteatus*, which despite naturally occurring in the Mediterranean basin, is not the best adapted to the conditions found in its agricultural areas. Sphaerophoria 17 18 rueppellii is another common hoverfly species in the Mediterranean area both in outdoor and 19 greenhouse crops. However, little information about the biology of this species is known and 20 this is one of the main requirements for every control-based IPM program. In this work we assess the influence of different humidity and temperature combinations on the performance 21 22 (mortality, developmental time and sublethal effects) of S. rueppellii under controlled 23 conditions. We also study the effect of aphid supply during larval stage on mortality and 24 developmental time of immature stages and on sublethal effects on adults. The results show 25 that this species requires high environmental humidity (higher than 60%) to complete its 26 development. Also, S. rueppellii is able to properly develop at a wide range of temperatures 27 (20-30°C). Sphaerophoria rueppellii reduces its voracity under low aphid density conditions, being able to adapt its feeding rate to the aphid availability. Mortality was not affected by 28 food supply, while low prey availability led to longer developmental time and a decrease in 29 30 larval and pupal weight and adult body size. These results show the suitability of this species as an aphid biological control agent under high humidity and temperature conditions, such as 31 32 the ones inside Mediterranean greenhouses.

33 Keywords:

34 Syrphidae, temperature, relative humidity, life cycle, aphid supply, greenhouse

35 1- INTRODUCTION

The Mediterranean basin has the highest horticultural crop production of Europe (FAOSTAT, 2009), and the surface devoted to organic and integrated production increases every year, particularly in protected (i.e. greenhouse) crops (Freier and Boller, 2009). Horticultural crops have several key pests, aphids (Hemiptera: Aphididae) being one of the most important. More specifically, polyphagous species such as *Aulacorthum solani* (Kaltenback), *Aphis gossypii* (Glover), *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae* (Sulzer) are the most damaging aphids in protected crops (Blümel, 2004; Rabasse and Steenis, 2002).

43 Several species of predators and parasitoids have been used in greenhouse crops 44 against aphids, providing unsatisfactory results that led to the use of compatible chemicals to 45 control this pest (Ramakers, 2004). In addition, biological control of aphids has been based on the same species for more than two decades, and only recently new and more-effective natural 46 47 enemies have started to be commercialized and released for aphid control (Sanchez et al., 48 2011). Several studies have proven that the extreme climate conditions inside greenhouse vegetable crops can influence the establishment and reproduction of natural enemies (Shipp 49 and Van Houten, 1997; Zhang and Shipp, 1998) as well as the development and survival of 50 51 their immature stages, preventing most of the species traditionally used from a proper performance. For these reasons, there is a strong demand for effective biological control 52 53 strategies against aphids on protected horticultural crops (Sanchez et al., 2011). Specifically, there is a current trend to encourage the search and use of indigenous natural enemy species, 54 55 that has already led to the replacement of several popular exotic biological control agents with 56 indigenous species (van Lenteren, 2012).

57 Aphidophagous syrphids (Diptera: Syrphidae) are high potential agents in aphid 58 biological control (Brewer and Elliott, 2004; Freier et al., 2007; Haenke et al., 2009; 59 Tenhumberg and Poehling, 1995), being also compatible and complementary with the use of

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other biological control agents, such as parasitoid wasps (Pineda et al., 2007) or entomopathogenic fungi (Diaz et al., 2010). However, there is only one commercially available species, *Episyrphus balteatus* (De Geer). This species is particularly abundant and efficient on central and northern Europe (Tenhumberg, 1995), but it is not the most abundant in Mediterranean agricultural areas (Pineda and Marcos-Garcia, 2008b). A high mortality rate when temperature is higher than 25°C has been reported (Hart et al., 1997), a temperature condition which normally occurs in outdoor and indoor crops.

Sphaerophoria rueppellii (Wiedemann) is a predator hoverfly which usually occurs in 67 68 Mediterranean crops (Pineda and Marcos-Garcia, 2008b; Speight, 2005). This species seems 69 to be the best adapted to the extreme climatic conditions of high temperature and humidity, 70 such as the ones occurring in Mediterranean areas, and particularly those taking place in greenhouses from southeastern Iberian Peninsula (Pineda and Marcos-Garcia, 2008b). In 71 72 addition, previous work has shown that habitat management strategies such as providing 73 additional floral resources (Pineda and Marcos-Garcia, 2008c) and banker plants (Pineda and Marcos-Garcia, 2008a) effectively enhance S. rueppellii's populations in sweet-pepper 74 Mediterranean greenhouses. Thus, this species has a high potential as an aphid biological 75 76 control agent in protected crops, and it has been suggested to make this syrphid commercially available (Pineda and Marcos-Garcia, 2008b). However, little information about the biology 77 of this species is known to date. 78

One of the first steps before natural enemies can be used effectively as mass reared biological control agents is to obtain valid information about their biology and their interaction with herbivore insects (Wiedenmann and Wilson, 1996). This work aims to provide information about some biotic and abiotic requirements of the aphidophagous syrphid *S. rueppellii*, one of the main requirements previous to rear this insect with commercial purposes. Our study system also consists of sweet-pepper [*Capsicum anuum* L. (Solanaceae)],

85 a horticultural species largely extended in the Mediterranean area (FAOSTAT, 2009), and the 86 aphid *M. persicae*, a species that causes severe economic losses in numerous crops worldwide 87 (Blackman and Eastop, 2000; Minks and Harrewijn, 1989). We assess the influence of different humidity and temperature combinations as well as of food supply during larval 88 stage, on the performance of immature S. rueppellii stages under controlled conditions as well 89 5 CP 90 as on sublethal effects on the developed adults.

91

2- MATERIAL AND METHODS 92

93 2.1- Plant material, aphids and syrphids

94 Sweet-pepper plants (C. annuum var. California Wonder) were grown from seed in a climate 95 room (T = $25^{\circ}C \pm 1^{\circ}C$, RH = $60\% \pm 10\%$, Photoperiod = 14L:10D, average light intensity = 495 lux) in plastic pots (5 x 5x 6 cm). These conditions were used throughout all of the 96 97 rearing in this work. A stock colony of *M. persicae* was maintained on sweet-pepper plants in 98 plastic boxes (30 x 30 x 60 cm) covered with a fine mesh on its upper part. The aphids were kept in a climate room with the same conditions as for plant growth. Sphaerophoria rueppellii 99 cohorts were reared in plastic cages (30 x 30 x 60 cm), with a fine mesh on its upper part. The 100 101 rearing cages contained bee granular pollen (© Sigma-Aldrich Co. LLC.), sugar provided as dry cubes, water (presented on a 100ml plastic glass inverted on a Petri dish lid with a disc of 102 103 filter paper) and a sweet-pepper plant infested with the aphid *M. persicae* to stimulate oviposition. Larvae were reared on *M. persicae* colonies on sweet-pepper plants. 104

105 **3.1-** Abiotic requirements

106 To assess the effect of environmental conditions on immature stages of this hoverfly, three 107 different temperatures (20°C, 25°C, 30°C \pm 1°C) and two relative humidity conditions (50%, 108 $90\% \pm 10\%$) were selected. Such conditions are common in both outdoor crops and

greenhouses throughout spring and summer for a wide range of latitudes. Six treatments wereestablished, representing all the possible combinations of those environmental conditions.

111 The temperature conditions needed were obtained by using three controlled FITOTRON cabinets, one for each temperature. Although this type of design mixes the effect 112 113 of temperature and cabinet, it is a common problem among temperature cabinet studies and it is difficult to solve. The high humidity treatments were kept by using Petri dishes with a wet 114 disk of paper inside and by sealing the dishes with Parafilm[®]. For low humidity treatments 115 Petri dishes with a net on its upper part were used, so the humidity conditions inside the dish 116 117 and that programmed on the rearing chamber were the same. Petri dish position was 118 randomized and changed daily to compensate for any directional bias from the cabinet 119 structure. Temperature and relative humidity inside the Petri dishes were checked by using Dataloggers (HOBO[®] U10-003). The Photoperiod throughout the experiment was 14:10 120 121 (L:D), and the average light intensity was 495 lux.

122 50 replicates were performed for each stage, consisting of a single egg, larva or pupa placed in a Petri dish (90 x 15 mm) with a piece of a sweet pepper leaf (1 cm²). Individuals 123 from one assay were not re-used in other stage-assay. Eggs with the same age were obtained 124 125 by placing an aphid-infested sweet-pepper plant in a rearing cage containing mature adults for two hours. Each egg was then removed from the plant by cutting off a small piece of plant 126 127 tissue with the egg to avoid damage, and placed separately in a Petri dish. Mortality rate and time to egg hatching were recorded by checking each replicate every 24h. Larval and egg 128 129 mortality could be determined visually, whereas pupal mortality was scored when no adult 130 emerged one week after the last adult emerged. A total of 50 eggs per treatment were 131 observed.

Focusing on the larval stage, Ad libitum food supply (*M. persicae*) was providedthroughout the larval cycle. Remaining aphids were removed daily and replaced with aphids

from a rearing under controlled conditions, to avoid a negative effect in their quality due to temperature and humidity conditions inside the Petri dishes. The piece of sweet pepper leaf was removed daily as well, to make sure that the aphids remained on it without spreading on the dish. Larval mortality and developmental time of larvae were assessed by checking each Petri dish every 24h, from a total of 50 larvae per treatment.

139 Once larvae pupated, the pupae were kept under the conditions of the corresponding 140 treatment. From the emerged adults, wing length was also determined, which is a good 141 indicator of total body size in syrphids and other diptera and so, a good indicator of fitness 142 (Stubbs and Falk, 1983). Specifically, the shortest length between the junction of the costal 143 vein (C) with the humeral crossvein (h) and the junction of R4+5 with the medial vein (M) 144 was measured (Dziock, 2005). Due to differences between males and females, measurements 145 were analyzed separately for each sex. Morphological measurements were always done using 146 the left wing, to avoid any effect due to asymmetry.

To study the effects on pupal stage, syrphid eggs and larvae were reared under the standard rearing conditions described above. Then each pupa was placed separately in a Petri dish, on a piece of leaf where the pupation occurred to avoid manipulation. Mortality rate and time of development were determined, as well as wing length of the emerged adults. A total of 50 pupae per treatment were observed.

152 **3.2- Biotic requirements**

To assess the effect of food supply during larval stage on the performance of this hoverfly, two treatments were established: The "high food availability" treatment, where a high number of aphids (*M. persicae*) were provided throughout the larval stage, and the "low food availability" treatment, where we provided larvae with a limited amount of aphids, enough to allow the larval stage to complete. The daily amount of aphids for each treatment (see Table 1) was based on a preliminary experiment where the maximum and minimum number of

aphids consumed by *S. rueppellii*'s larvae were determined (see Supplementary Table 1). We provided larvae from "high food availability" treatment with aphids according to the maximum daily aphid consumption data, while "low food availability" treatment was supplied with aphids according to the minimum daily aphid consumption obtained in this preliminary trial.

Each replicate consisted of a one-day old larva placed separately on its own Petri dish with a piece of sweet pepper leaf (1 cm²), a wet disk of paper and sealed with Parafilm[®], to achieve 90% RH inside. Most of the aphids remained feeding on the leaf, so even first instar larvae, with a limited movement capacity (Chandler, 1968) were able to prey on the aphids that they needed. 60 replicates were performed for each treatment.

169 Every 24 hours remaining aphids in the Petri dish were removed and counted. After that, new aphids from a rearing were provided. Only third and fourth instar-aphids were 170 171 provided, to avoid underestimating the predation owing to the potential progeny production 172 by aphid adults. Through observations every 24h, daily and total aphid consumption rate throughout the larval stage were determined, as well as mortality rate and time of 173 development of both larvae and pupae. Also, the third instar larvae and newly formed pupae 174 175 were weighed using a precision weighing scale (Acculab ALC-110.4 Analytical Balance). Pupae were placed in two 30 x 30 x 60 cm plastic rearing cages according to the treatment, 176 with a fine mesh on its upper part. Emergence rate and wing length of adults were measured. 177 178 Experiments were carried out with the same environmental conditions explained above.

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180 **3.3 – Statistical analysis**

181 The normality of the data was tested with the Kolgomorov-Smirnov and Shapiro-182 Wilk tests. Data on mortality rate, developmental time and aphid consumption were not 183 normally distributed and therefore were analysed with Generalized Linear Models (GENLIN)

184 followed by LSD post-hoc test for multiple pair-wise comparisons. The model was adjusted to 185 a Poisson distribution with log link function for developmental time and aphid consumption, 186 and to a binomial distribution with logit link function for mortality. Data on aphid consumption was analyzed with GENLIN for repeated measures (days). Data on wing length 187 188 and weight were normally distributed and therefore were analyzed with a t-test or ANOVA, 189 followed by Tukey post-hoc test for multiple pair-wise comparisons. Wing length data from 190 the larvae experiment were analysed with a one-way ANOVA to study the differences among temperatures, due to the fact that survival of larvae was 0% in the 50% RH treatments. Data 191 192 on wing length from the pupae experiment were evaluated with a two-way ANOVA to 193 determine effects of relative humidity and temperature. All data were analysed with the statistical packages SPSS V19.0 and Gen Stat 13. 194 NP

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196 **4- RESULTS**

197 **4.1-** Abiotic requirements

Low humidity had a strong negative impact on the performance of S. rueppellii. Eggs (Fig. 1) 198 and larvae (Fig. 2) were the most sensitive stages, with a higher mortality (egg: df = 1, 299; 199 200 deviance ratio = 97.58; P < 0.001; larva: df = 1, 299; deviance ratio = 781.79; P < 0.001) and longer developmental time (egg: df = 1, 151; deviance ratio = 203.76; P < 0.001) when 201 202 developing at 50%RH compared to 90%RH. Actually, larval mortality was 100% for all 203 treatments when developing at low humidity, which made impossible to obtain data of their 204 developmental time as well as pupa and adult parameters for these treatments. Interestingly, 205 pupae were not affected by low humidity conditions (df = 1, 299; deviance ratio = 2.26; P >206 0.05; Fig. 3).

207 The increase of temperature had no effect on egg and larva mortality (egg: df = 2, 299; 208 deviance ratio = 2.13; P = 0.121; larvae: df = 2, 299; deviance ratio = 3.02; P = 0.051),

whereas at 30 °C pupal mortality significantly increased compared with the other 209 210 temperatures (df = 2, 299; deviance ratio = 5.81; P = 0.003; LSD: 20-30°C P = 0.003, 25-211 30°C P = 0.02). Interestingly, when larvae developed at 25°C or 30°C, the following pupal stage also had significantly higher mortality compared with larvae that developed at 20°C (df 212 = 2, 149; Wald statistic = 7.213; P = 0.03; LSD: 20-25°C P = 0.026; 20-30°C P = 0.008) (see 213 Supplementary Fig. 1). In contrast, temperature had a positive effect on the developmental 214 215 time of the three S. rueppellii stages, which showed a faster development as the temperature raised (egg: df = 1, 151; deviance ratio = 203.76; P < 0.001; larvae: df = 2, 130; Wald statistic 216 217 = 194.5; P < 0.001; pupae: df = 2, 276; deviance ratio = 1293.58; P < 0.001; LSD, P < 0.001). 218 However, the faster larval development had a negative consequence for the body size of the emerging adults, since the increase of temperature reduced wing length of males ($F_{2, 20}$ = 219 31.46; P < 0.001) and females (F_{2, 22} = 31.01; P < 0.001 between 20°C and 25-30°C, P < 0.05220 221 between 25°C and 30°C). Similarly, faster pupal development led to shorter wing length in 222 both males (df = 2, 89; F = 44.85; P < 0.001) and females (df = 2, 89; F = 48.48; P < 0.001) suggesting that this reduction in body size is independent of a lower food intake. 223

224

225 4.2- Biotic requirements

The daily feeding rate of larvae (Fig. 4) was affected both by the level of food supply (df = 1, 226 227 1115; Wald statistic = 21.5; P < 0.001) and by the day of development (df = 11, 1115; Wald 228 statistic = 7618.8; P < 0.001). The maximum aphid consumption was achieved 2 days later on 229 the low food availability treatment, and the developmental time was also 2 days longer in this 230 treatment (Fig. 4). A limited supply of aphids, had no impact on larval (df = 1, 89; Wald 231 statistic = 0.0001; P = 0.992) nor pupal (df = 1, 67; Wald statistic = 1.323; P = 0.254) 232 mortality of S. rueppellii (Fig. 5A). However, limited food supply increased the 233 developmental time of larvae (Fig. 5B) compared with the high food availability treatment (df

234	= 1, 92; Wald statistic = 140.7; $P < 0.001$), whereas pupal developmental time remained
235	unaffected (df = 1, 86; Wald statistic = 0.192; $P < 0.663$). As we expected, limited food
236	supply had a negative effect on the weight (Fig. 5C) of third instar larvae (df = 53, t = 4.71, P
237	< 0.001), and newly formed pupae (df = 68, t = 4.81, $P < 0.001$). In the same way, the wing
238	length of both adult females (df = 17, t = 2.49, $P = 0.023$) and males (df = 15, t = 3.39, $P =$
239	0.004) decreased with limited food supply during larval stage (Fig. 5D).
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241	5 – DISCUSSION
242	5.1. Abiotic requirements

240

5 – DISCUSSION 241

242 **5.1.** Abiotic requirements

The results of this study show that abiotic conditions have a key role on the development of 243 244 different immature stages of S. rueppellii. Pineda and Marcos-García (2008b) evaluated the composition and population dynamics of aphidophagous syrphids inside sweet-pepper 245 greenhouses from southeastern Spain between 2004 and 2006. They found out that S. 246 247 rueppellii was much more abundant than the rest of species in the years when drought periods happened earlier and lasted longer, suggesting that this species was the best adapted to the dry 248 conditions occurring in Mediterranean areas compared with the other syrphid species 249 250 identified, including E. balteatus and Eupeodes corollae (Fabricius). Additionally, such study showed that S. rueppellii appeared later in the season than the other syrphid species, when 251 252 temperatures were higher. Based on that study, we expected that the performance of S. rueppellii would improve at high temperature and low humidity. In contrast to our initial 253 254 expectations, our results do not support the hypothesis that S. rueppellii develops better under 255 dry conditions. Specifically, this study shows that egg and larval stages are particularly 256 susceptible to low relative humidity, whereas pupal stages are not affected by the humidity 257 conditions (measured as developmental period, mortality rate and adult's wing length). However, the potential of this species to naturally establish on protected crops and efficiently 258

control aphid populations would be favoured by the high humidity conditions occurring insidegreenhouses.

261 Hoverflies may have several strategies to tolerate drought conditions in the field, such as the use of microhabitats or the adjustment of their activity schedules. Syrphid larvae 262 263 are generally more active during the night than in daylight (Holmes, 1985; Vickerman and 264 Sunderland, 1975), although we have observed in the field that S. rueppellii has also a 265 voracious predatory activity during the day. On the abaxial (lower) surface of leaves, which 266 cannot be directly reached by light, a higher relative humidity and lower temperature occurs 267 compared with the adaxial (upper) surface because of the higher stomata density (Gutschick, 268 1999). Personal observations both in laboratory and field conditions indicate that S. 269 rueppellii's females lay their eggs preferably on the reverse of leaves and larvae can normally 270 be found here and on other areas of the plant where the humidity has been condensed. 271 Regarding the pupal stage, almost all the pupation observed in controlled conditions takes 272 place in plant areas with a high condensation, such as the back of the leaves, places where a 273 leaf is on top of the other, dead leaves or roots and fibres present on the substrate. These observations suggest that under low humidity conditions, like the ones occurring in outdoor 274 275 crops or outside greenhouses, S. rueppellii could exploit these microhabitats where the optimal humidity and temperature conditions for its development and activity may be 276 reached. 277

Pupa is the only stage for which mortality is affected by temperature independently of the relative humidity, increasing when the pupa is exposed to higher temperatures. This indicates that, although pupal developmental time and mortality are not affected by relative humidity, this immature stage is the most sensitive to the surrounding temperature. Development of all immature stages was completed faster when the temperature rose, with the shortest developmental period taking place at 30°C. Honek and Kocourek (1988) and Saidov

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(1974) found this negative relation between temperature and developmental period in more
than 20 species of aphidophagous insects, including syrphids such as *E. balteatus* and *Sphaerophoria scripta* (L.).

The relation between body size, fitness and temperature has been documented on a 287 288 high number of animals, especially on insects (Atkinson, 1994), and it can be summarised in two rules. The first rule 'hotter is smaller' proposes that ectotherms that develop at higher 289 temperatures will be relatively small as adults (Angilletta and Dunham, 2003; Atkinson, 290 291 1994), and our results on wing length support this pattern. The second rule 'Bigger is better' 292 proposes that individuals with larger body size will tend to have greater performance and 293 fitness than smaller individuals (Bonner, 2006; Peters, 1983) in the sense of a greater survival, 294 fecundity and mating success (Kingsolver and Pfennig, 2004). According to this rule, our results of wing length suggest a negative effect of the temperature increase on S. rueppellii 295 performance, as the mortality results also show. Nevertheless, some authors (Frazier et al., 296 297 2006; Savage et al., 2004) suggest that there is another rule (*Hotter is better*), proposing that species that have adapted to high optimal temperature can also have enhanced fitness at high 298 temperatures, through a shortened generation time (Roff, 2002). Interestingly, our results also 299 300 support this hypothesis, since the three larval stages developed faster with increasing temperatures. Since S. rueppellii provides a case of a species that has evolutionarily adapted 301 302 to warm climate, it will be an excellent candidate to evaluate whether the benefit of shorter developmental time caused by high temperatures overrides the fitness cost of the decrease in 303 304 body size.

Immature stages of *E. balteatus*, the only syrphid species in commercial production, suffer a high mortality with temperatures over 25°C (Hart et al., 1997). In previous studies, we monitored the temperature in several commercial greenhouses of southeastern Spain during two years, and temperatures in spring varied between 11°C (during night) and 38°C (in

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309 daytime). We believe that S. rueppellii is a high potential control agent on Mediterranean 310 greenhouses, due to a better tolerance to the high temperatures present on these environments. 311 Despite there are evidences that S. rueppellii's performance decreases when developing at high temperatures, this is the most abundant species in Mediterranean greenhouses towards. 312 313 the end of spring, when the daily maximum temperature is over 25°C, indicating that out of the species recorded, this hoverfly is the best adapted to the high temperature conditions 314 315 present inside greenhouses (Pineda and Marcos-Garcia, 2008b). Augmentative biological control is an important part of IPM programs, and the availability of several natural enemies 316 317 that are effective under different abiotic conditions will contribute to the success of pest 318 control under a changing climate. In addition, the use of native species and the high 319 specificity of aphidophagous hoverflies ensures a lack of side effects to the environment and negative intraguild interactions with other natural enemies, which is another key point for 320 321 satisfactory results of biological control strategies.

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323 5.2- Biotic requirements

Ovipositional preference by females has a profound effect on the performance of their 324 325 offspring, ensuring the newly emerged larvae have enough food to develop (Sadegui and Gilbert, 2000). However, some indications imply that even small syrphid larvae must move 326 327 between plants in search for new aphid colonies (Banks, 1968) as a single aphid colony may be insufficient to support larval development to maturity (Kan, 1988a; Kan, 1988b). 328 329 Sphaerophoria rueppellii is able to complete its life cycle even under low aphid availability 330 conditions, with survival of larvae not being affected by diet. This fact could allow the larvae 331 to exploit small and developing aphid colonies, moving among different plants or areas within 332 a plant, which is a very desirable feature on a biological control agent. Several authors state 333 that aphid quantity and quality is a major factor influencing the development time of

334 aphidophagous syrphids (Dziock, 2005; Polak, 1980; Rojo et al., 1996). Limited food supply 335 increases the developmental time of S. rueppellii's larvae while, interestingly, the 336 developmental period of pupal stage does not change in accordance with aphid's consumption throughout larval stage. This trend has also been observed on the hoverfly E. corollae, while 337 338 in other species such as E. balteatus, pupal development shortens when larvae are provided 339 with excess of aphids (Rojo et al., 1996). Nevertheless, a reduced weight of immature stages 340 and wing length of adults is reached when aphid supply is not high enough. Consequently, larvae's diet has a major effect on body size of S. rueppellii, like has been reported on other 341 342 hoverfly species like E. corollae and E. balteatus (Rojo et al., 1996). Body size is correlated 343 with a large number of species' attributes that influence their fitness, including longevity, 344 reproductive rate or resource use (Blackburn and Gaston, 1994; Harvey and Pagel, 1991).

Daily feeding rate under high food availability conditions reaches a peak once larvae 345 have reached the third instar. Adams et al. (1987) and Soleyman-Nezhadiyan and Laughlin 346 347 (1998) found that the last larval instar of syrphids is also the most voracious, been responsible of up to 90% of the total aphid consumption. Mean daily consumption under high food 348 availability conditions was $26,11 \pm 4,89$ aphids/day, a slightly lower value than the one 349 350 measured by Ming Hong and Quang Hung (2010) with the species E. balteatus feeding on M. *persicae* $(31,4 \pm 1,88 \text{ aphids/day})$. Under low food availability conditions, the maximum 351 352 aphid consumption of S. rueppellii's larvae is postponed two days, since third instar is reached later than in optimal food conditions. Our results agree with what has been exposed 353 354 by several authors, which assure that the proportion of prey consumption during the first few 355 days after eclosion is small (Adams et al., 1987; Barlow, 1979), and it decreases again 356 towards the end of larval stage (Tenhumberg and Poehling, 1995).

357 Although the developmental period of the larvae is two days longer with limited 358 aphid supply, the total aphid consumption is higher under high food availability conditions,

359 being able to prey on nearly 220 aphids throughout this stage, a higher value than the one 360 found by Hopper et al. (2011) with a species from the same genera, Sphaerophoria 361 sulphuripes (Thomson) with a lifetime consumption of 194.44 third instar aphids. Even though aphid supply on the Low food availability treatment was below the optimal, S. 362 363 rueppellii's larvae hardly consumed all the aphids provided. The average number of nonpreyed aphids that remained on the Petri dishes was 7.61 individuals (30% of the aphids 364 365 supplied). A possible explanation to this may be that at very low aphid densities S. rueppellii's larvae could be able to adapt their feeding rate according to the food availability 366 367 conditions, as found by Tenhumberg and Poehling (1995) on some syrphids such as E. 368 balteatus and S. scripta.

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370 6. CONCLUSIONS

Because aphid populations grow exponentially, biological control strategies by using 371 372 hoverflies should achieve a greater impact on aphid population during an early stage of the infestation (Tenhumberg and Poehling, 1995). Under low aphid density conditions, sublethal 373 effects are observed on the S. rueppellii adults developed in such conditions, highlighting a 374 375 reduced fitness in such conditions. According to this, the use of inoculative releases would not 376 be effective, because under persistent low food availability conditions, the number of syrphids 377 might not be sufficient to sustain a permanent population in the field and new releases would 378 be needed in order to keep controlling the pest population. On the other hand, the fact that 379 mortality rate of larvae does not increase even under low aphid density conditions, supports 380 the potential use of this predator on augmentative control strategies through inundative 381 releases. In addition, the fact that this species is adapted to the high humidity and temperature 382 conditions similar to those occurring in Mediterranean greenhouses makes this species even 383 more suitable for its use in these environments. However, estimating predatory efficiency of

syrphid larvae based on laboratory estimates may lead to an overestimation on their actual impact on the pest in the field (Tenhumberg and Poehling, 1995). Additional factors may affect the efficacy and population dynamics of predators in field conditions, such as intraguild predation (Rosenheim et al., 1995), variation of environmental conditions and prey quality (Symondson et al., 2002), sublethal effects of pesticides (Desneux et al., 2007) or habitat management strategies (Landis et al., 2000), pointing out that field studies are needed in order to evaluate *S. rueppellii*'s effectiveness as an aphid control agent in commercial greenhouses.

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530 Figure legends

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Figure 1: Abiotic requirements for eggs. A) Mortality rate (mean %). B) Developmental

time (mean + SEM). Different letters above bars indicate significant differences (GENLIN,

534 LSD).

Figure 2: Abiotic requirements for larvae. A) Mortality rate (mean %). B) Developmental
time (mean + SEM). C) Wing length of adults (mean + SEM). Different letters above bars
indicate significant differences (GENLIN, LSD for mortality and developmental time,
ANOVA and Tukey HSD for wing length).

Figure 3: Abiotic requirements for pupae. A) Mortality rate (mean %). B) Developmental
time (mean + SEM), C) Wing length of males and females (mean + SEM). Different letters
above bars indicate significant differences (GENLIN, LSD for mortality and developmental
time, two-way ANOVA and Tukey HSD for wing length).

Figure 4: Daily aphid consumption rate (mean + SEM) throughout larval stage on high and
low food availability treatments. Data were analyzed with a GENLIN for repeated measures.

Figure 5: Performance with different aphid supply. A) Developmental time of larvae and pupae (mean + SEM). B) Mortality rate of larvae and pupae (mean %). C) Weight of larvae and pupae (mean + SEM). D) Wing length of adults (mean + SEM). ns = not significant; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001 (GENLIN for developmental time and mortality, T-test for independent samples for body weight and wing length).

Supplementary Figure 1: Mortality rate of resulting pupae (mean %). Different letters
above bars indicate significant differences (GENLIN, LSD).

- 552 Sphaerophoriarueppellii: an indigenous predatory hoverfly in the Mediterranean basin
- 553 Low relative humidity strongly impacts survival of larvae and eggs, but not of pupae
- 554 Development is completed at a wide range of temperatures, even at 30°C
- 555 Survival of immature stages is not affected by aphid supply
- Acception

Treatment/Day	1	2	3	4	5	6	7	8	9	10	11
Ad libitum	10	20	30	50	60	65	65	70	70	70	70
Limited food	1	5	5	15	25	25	35	35	35	35	35
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Abiotic requirements



Optimal development of eggs and larvae at high relative humidity and temperature

> Pupae highly resistant to abiotic stress